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(71) Applicant: **SNOW BRAND MILK PROD CO LTD**

(72) Inventor: **SHIIKI YASUHIKO**
HIGASHIMURA MAKOTO
ITO KENSUKE
KAMEOKA KOJI

(54) **FERMENTATION CONTROL AND MEASUREMENT OF ACIDITY OF LACTIC ACID BY USING INFRARED ATR SPECTROSCOPY**

(57) Abstract:

PURPOSE: To accurately control the state of the fermentation by measuring infrared absorption, etc., of the fermentation broth at a specific infrared absorption frequency of a component whose concentration changes in the course of the fermentation by microorganism.

CONSTITUTION: Infrared absorption or a change of infrared absorption of a fermentation broth at a specific infrared absorption frequency of a component (a sugar, an alcohol, etc.), whose concentration changes in the course of the fermentation by microorganism, is measured. Infrared attenuated total reflection spectroscopy (IR-ATR) enables the easy separation of absorption of water from that of the material to be measured and the amount of the material is qualitatively measured without receiving the influence of water.

* NOTICES *

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DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Industrial Application]This invention relates to the control method of the fermentation by microorganisms which used the infrared attenuated total reflectance method (infrared ATR method), such as yeast and lactic acid bacteria. Furthermore, this invention relates to the measuring method of the lactic acid acidity in the fermented mash in lactic acid fermentation.
[0002]
[Description of the Prior Art]The fermentation using a microorganism is production of drugs and an industrial source, etc. from foodstuffs. Usually, control of these fermentation is performed, monitoring the quantity of production of the quality of an object by measurement by an in-line sensor, analysis by batch type sampling, etc. Although it is common to control a fermentation state especially in production of a fermented food by making into an index organic acid, alcohol, etc. which are the main products produced by fermentation, it may control by replacing with measurement of such main products and measuring pH, dissolved oxygen concentration, turbidity, etc. Especially the fermentation that used lactic acid bacteria or yeast also in the fermented food is used for manufacture of foodstuffs, drinks, etc. for many years. For example, fermented milk, a lactic acid bacteria beverage, etc. are the typical examples of a product of the fermentation which used lactic acid bacteria, and beer, wine, Japanese sake, etc. are the examples of a product of the fermentation which used yeast.
[0003]As the manufacturing method of lactic fermentation food and a drink is described below, it is generally performed. If it is in the lactic fermentation food which used milk as the raw material, powdered skim milk or concentrated milk is added to cow's milk and skim milk, Preparation mixture of the auxiliary material is carried out, uniformity and germicidal treatment are performed for this in accordance with a conventional method, a mixed lactic starter or an independent starter is inoculated 2 to 3% after cooling at 30-50 **, lactic acid fermentation is kept warm and carried out to 30-40 **, and it is manufactured. At this time,

the stop of fermentation is usually performed by cooling or heat-sterilizing a fermentation substrate (prepared solution which added milk, skim milk liquid, or an auxiliary material in the above-mentioned case). Generally the judgment of the timing of the fermentation stop the lactic acid generated by lactic acid fermentation, The measurement or pH of lactic acid acidity by the titration method using the alkali reference solution (usually sodium hydroxide solution) in which that concentration is measured beforehand was measured, the correlation of this measurement pH and acidity was searched for beforehand, and it has determined from this correlation. However, the method of judging a fermentation stop by these measurement has faults, like the influence by temperature is that there is dispersion according [measurement] to an operating personnel, unsuitable for in-line measurement, and great. In the method of judging a fermentation stop by lactic acid acidity measurement especially, since measurement operation was complicated, measuring time took the long time, fermentation progressed between the measurement, the timing of the stop separated, and it often became fault fermentation. Although the method of installing a pH sensor in a tank and monitoring the pH change as a method of monitoring a fermentation process with in-line one is also tried in lactic acid fermentation, The outflow from the structure of a pH electrode to the inside of the substrate of the internal liquid of an electrode was not avoided, but was unsuitable as an in-line measuring method for food manufacturing.

[0004]On the other hand, in alcoholic fermentation, it carried out as follows. That is, although various fermentation gestalten are taken with a product, the mash which generally added the yeast starter to the saccharified raw material is fermented, usually when 5 to 20% of alcohol concentration is reached, fermentation is terminated, and mash separation is performed. In this case, although it filtered by having extracted mash and this filtrate was analyzed, filtration operation and analysis took time and there was a problem of being unable to perform suitable fermentation management.

[0005]There is almost no example which uses a pH sensor by fermentation control of foodstuffs. Although the fermentation tube law using an electromagnetic induction type conductivity meter is shown by JP,2-9780,B as a means to solve the above-mentioned problem, By this method, the influence by the temperature of conductivity is great, the value of conductivity changes greatly with substrate ingredients, and there is a fault of correspondence with the conventional lactic acid acidity not being decided uniquely. Furthermore, By near-infrared spectrometry. Lactic acid fermentation. The method of managing was also proposed (Giuseppe.). Vaccari and others, "A Near-Infrared Spectroscopy Technique for theControl Fermentation Process: An Application to Lactic Fermentation", Biotechnology andBioengineering, Vol. 43, p.913-917, and 1994, This method had the fault that it had to ask for regression with control values, such as an absorbance and lactic acid acidity, again, when substrate ingredients differed for every fermentation.

[0006]Although there is the method of stirring after making it ferment in the method of fermenting within a container and a tank, and using as a product in the manufacturing

method of yogurt, It is necessary to monitor the fermentation state in a container for example, and, in the case of the former, to JP,2-236141,A. After having fermented, putting the foodstuffs to solidify into the container and carrying out a seal, the method of carrying out damped free vibration of the whole container, measuring the cycle or the number of damped free vibration, and amplitude of damped free vibration, and controlling fermentation was indicated, but it was impossible to have set up the timing of a fermentation stop arbitrarily in this method. In alcoholic fermentation, measured the carbon dioxide and ethyl alcohol gas which are produced with fermentation with the infrared analyzer, and the method of controlling the fermentation state of fermentation mash was proposed so that it might be indicated by JP,60-149374,A, but. Here, the gasified ingredient is measured, change of the component amount contained in mash is measured, and a fermentation state is not controlled. As mentioned above, there are many problems in the control method of the fermentation by an old microorganism, and the method of controlling a fermentation state with sufficient accuracy was not proposed.

[0007]

[Problem(s) to be Solved by the Invention]When this invention persons consider infrared absorbance analysis and the application to the fermentation control is considered, it is a unique infrared-absorption wave number of the ingredient which carries out a concentration change by a metabolic turnover in the fermentation process by a microorganism, While finding out that it was possible to check change of a fermentation state by measuring an infrared absorbance and measuring this absorbance or the amount of absorbance variations for the first time, change of the absorbance in this unique infrared-absorption wave number found out having a fermentation product in fermented mash, and strong correlation. Offer of the control method of the fermentation by this invention having been made based on such knowledge, and measuring the fermentation process by a microorganism directly using infrared absorbance analytical method is a technical problem. Let it be a technical problem to provide how this infrared absorbance analysis performs fixed-quantity measurement of the specific component in fermented mash.

[0008]

[Means for Solving the Problem]In infrared absorbance analysis, it can ask for a specific absorption wave number of each substance by conducting the absorption-spectrum analysis. Sugar, alcohol, stretching vibration of C-OH origin [at organic acid] in 1200 - 1000 cm^{-1}, Inverse symmetry elasticity of C-O-C origin in 1150 - 1070 cm^{-1}, Stretching vibration of C-O origin in 1085 - 1050 cm^{-1}, stretching vibration of C-O origin in 1250 cm^{-1}, Unique absorption based on C-O is observed in 1200 - 1040 cm^{-1} as well as [stretching vibration of C-O, and 925 cm^{-1}] stretching vibration of a C-O skeleton, and 1040 cm^{-1}. Thus, a wave number field of 900 - 1200 cm^{-1} is an absorption wave number common to sugar, alcohol, and organic acid, and this wave number field is called a fingerprint region of these substances. However, although using this wave number field for a quantitative

analysis of a substance was not performed until now, this invention persons already did patent application of this fingerprint region as Japanese Patent Application No. No. 52663 [six to] paying attention to using for a fixed quantity of a substance, especially sugar. As a result of inquiring further about a fingerprint region of this infrared absorption, this invention persons found out that change of absorption maximum in a fingerprint region was applicable to fermentation control, and completed this invention.

[0009]this invention method measures an infrared absorbance of a solution under fermentation with an infrared spectrophotometer, A difference of an absorbance with measured value before a fermentation start is searched for, it asks for a correlation formula of change of lactic acid acidity, and change of an absorbance, and a case of a fermentation control value which measured this measured value beforehand, for example, lactic acid fermentation, tries to get to know a fermentation state from an absorbance variation. According to this invention method, in a solution under fermentation, for example, lactic acid fermentation, a yogurt mix can control fermentation easily also in a solution in which multicomponent like mash under fermentation exists in alcoholic fermentation, respectively. However, by transmission type analytical method adopted from the former, in order to carry out a quantitative analysis except for influence of water by an infrared-absorption-analysis method, although it is difficult, separation of an absorption wave number of water absorption and a substance which it is going to measure becomes easy by using an infrared ATR method. It became possible to take out absorption of a fingerprint region which separated and mentioned water absorption above by adoption of such an infrared ATR method, and a fixed quantity of a substance became possible. A thing which measured by an infrared ATR method to drawing 1 and drawing 2 and for which an absorption spectrum in the air is separated although an absorption spectrum of water and glucose solution is shown, respectively is preferred. A wave number field of 900-1200 cm^{-1} is unique absorption of sugar, alcohol, and organic acid origin, and it becomes possible to measure change of sugar and alcohol which change by a fermentation process, and organic acid by measuring this absorption so that this spectrum may see.

[0010]As an absorbance of an absorption spectrum of this field was shown in drawing 4, it decreases in connection with a fermentation process in a fermentation solution, but this invention persons find out this phenomenon for the first time. Although it is thought that reduction of an absorbance of an absorption spectrum of this field corresponds with reduction of a substrate in a fermentation process, a decrement of this absorbance has a generated amount of advance of fermentation, i.e., fermentation metabolite made into the purpose, and strong correlation. By namely, a thing for which a linear expression which measured a concentration change of metabolite in a solution and metabolite accompanying advance of an absorbance in a specific wave number and fermentation beforehand, and measured a relation with reduction of concentration and an absorbance is stood, and an absorbancy index of the wave number is calculated. Quantity of metabolite can be known and a state of fermentation can be controlled from quantity of metabolite. Beforehand, if it

puts in another way, if an absorbancy index for every [in arbitrary wave numbers] ingredient is calculated by an infrared ATR method, by an infrared ATR extinction method, it becomes possible to ask for concentration of metabolite, and a fermentation state can be easily controlled only by measuring a fermentation solution in the middle of fermentation as a result. If measurement by infrared ATR in this invention is applicable to measurement of a fluid, it is usable no matter it may carry out with what device. Although it shows drawing 3 a typical structure, if measurement of a solution is possible for it even if an ATR absorption cell used by this invention method is a cell of structures other than this, it is usable in it. an infrared ATR analysis apparatus -- distributed type and a Fourier transform type -- although it may be any, especially a Fourier transform type especially is preferred. By incorporating such an infrared ATR analysis apparatus all over a factory line, it is also possible to consider it as an on-line nondestructive analysis measuring device.

[0011]A method of controlling a fermentation process by absorbance analysis using an infrared ATR device does not have a report or having been announced until now, and is a completely new idea. Although an example of this invention shows an example of lactic acid fermentation, If a substrate which has a unique absorption wave number in a fingerprint region of 900-1200 cm^{-1} is metabolized and reduction of this unique absorption has correlation with an increase in metabolite even if it is fermentation other than lactic acid fermentation, measurement and fermentation control are possible by this method. As an example of fermentation which such this invention method can apply, fermentation control in a case where various organic acid, such as lactic acid, acetic acid, and citrate, is produced, a case of performing alcoholic fermentation, such as ethanol, by using sugar as a substrate, etc. is raised by using sugar, such as glucose, milk sugar, sucrose, starch, and maltose, as a substrate.

[0012]When lactic acid fermentation from milk sugar is taken for an example, milk sugar which exists in a substrate is incorporated into intracellular, a beta galactosidase decomposes into glucose and galactose, and lactic acid bacteria perform lactic acid fermentation through an anaerobic metabolic fate, and make lactic acid of tetrad generate from one molecule of milk sugar theoretically. This quantitative relation is not materialized strictly in practice, but has some which pass along a course to minute amount metabolite, such as diacetyl, and this quantitative relation changes with kinds of bacillus. However, if relation between lactic acid acidity as a fermentation control value in lactic acid fermentation using a fixed starter or a lactic-acid-bacteria stock and sugars concentration, such as consumption milk sugar in a substrate, turns into linear relation and change of this sugars concentration is measured by an infrared ATR method, This absorbance or the amount of absorbance variations can be used as a fermentation control value. This absorbance variation is convertible for a fermentation control value like the conventional lactic acid acidity. Measuring extremely for a short time is possible, and short-time highly precise fermentation control of measurement by such an ATR method is attained as a result. That is, primary correlation is materialized between an absorbance variation which

was produced by doing in this way in the case of lactic acid fermentation, and lactic acid acidity, and it becomes possible to monitor a fermentation process by measurement of an infrared absorbance, and to control by asking for an absorbance variation, since lactic acid acidity can be determined in a short time.

[0013]Control of alcoholic fermentation by yeast which metabolizes sugar to alcohol can be performed similarly. In measurement, a fermentation solution is beforehand measured with the conventional fermentation control index, and an absorbance variation is measured by an infrared ATR method about the same sample. That is, the change of an absorbance can measure an absorbance before starter inoculation, and it can ask for change of an absorbance by deducting from an absorbance of each fermentation time. Since it expresses as absolute magnitude change of an absorbance of the whole fingerprint region, change of an absorbance is good also considering correction value based on minimum absorption wave number [of 960 cm] $^{-1}$ of a fingerprint region, and 1184 cm^{-1} as an absorbance, as shown in the following examples. Usually, what is necessary is just to amend an absorbance of a wave number used for measurement as a difference from average absorption of this minimum absorption. Although an example is shown below and this invention method is explained to it still in detail, this invention is not limited to a method of an example.

[0014]

[Work example 1]By this example, this invention method shows that it can apply to production control of fermented milk by lactic acid fermentation which used a mixed starter of Lactobacillus bulgaricus and Streptococcus thermophilus. A 1600 type Fourier-transform-infrared-spectroscopic-analysis meter by PerkinElmer, Inc. was used for an infrared-spectroscopic-analysis meter, and it used a device made from GRASEBY SPECIAL LIMIT which attached a ZnSe glass cell for fluid measurement for this device as an ATR apparatus. This cell is the thickness of drawing 3. A thing of 3.8 mm, 59.2 mm in length, and a 45-degree cut was used. A computer (SONY RCT-300) was connected with this, and IR data manager (PERKIN ELMER IRDM) was used and measured for software. Measurement was performed at temperature of 25 degreeC. Under these conditions, a water absorption spectrum was beforehand measured by an infrared ATR method. Subsequently, 14% 12% 10% to each reduction powdered skim milk of weight concentration. A mixed starter of Lactobacillus bulgaricus and Streptococcus thermophilus was added so that it might become 3% of weight concentration, and a differential spectrum which deducted a water absorption spectrum from an absorption spectrum was measured. Reduction powdered skim milk of each concentration was fermented in 37 degreeC within a thermostat. It sampled for every hour, lactic acid acidity which is a fermentation control value of lactic acid fermentation was measured with a titrimetric method by sodium hydroxide standard solution, a differential spectrum was simultaneously measured by an infrared ATR method, and aging of a differential spectrum in a fingerprint region was shown

in drawing 4. The highest peak in this differential spectrum is near the 1075 cm^{-1}, and this peak originates in stretching vibration of C-O-C of sugars. Furthermore with a differential spectrum, it has the minimum absorption in wave number 960 cm^{-1} and 1186 cm^{-1}, and asked for an amendment absorbance of 1075 cm^{-1} from an absorbance at this time. This amendment absorbance R_{1075} made an amendment absorbance of 1075 cm^{-1} R_{1075}, and asked for it by the several 1 following formula.

[0015]Namely, this amendment amends the absorbance of wave number 1075 cm^{-1} which is the maximal value of an absorbance by making into a baseline the line which connects two points of each minimal value of the absorbance of 960 cm^{-1} in Drawing 4, and 1186 cm^{-1}.

[Equation 1]

$$R_{1075} = \frac{(A_{960} - A_{1186}) \times (960 - 1075)}{960 - 1184} + (A_{1075} - A_{960})$$

[0016]A in a formula shows an absorbance. The relation between this amendment absorbance R_{1075} and the measured lactic acid acidity is shown in drawing 5. Amendment absorbance R_{1075} of a sample and lactic acid acidity which use the reduction powdered skim milk in each concentration as a substrate were in linear relation, and the inclination was not based on the concentration of reduction degreasing powder, but became a fixed value. From this result, the coefficient of one following formula showing change of this infrared absorbance and the relation of the variation of lactic acid acidity is obtained from the several 2 following formulas.

[0017]

[Equation 2]

乳酸酸度変化量 = -28.5445 × R_{1075}変化量

The coefficient of several 2 formulas [-28.5445] is common inclination of three straight lines shown in drawing 5.

[0018]Since amendment absorbance R_{1075} and lactic acid acidity are in linear relation, if the inclination is computed beforehand, milk sugar is consumed by lactic acid fermentation from R_{1075} variation, and they can control directly the process metabolized by lactic acid. This shows that it can ask for lactic acid acidity from the number 2 above-mentioned formula by measuring an infrared absorbance. That is, if it can ask for one following formula of an infrared absorbance and lactic acid acidity even if the system of production by fermentation changes, fermentation control can be performed from R_{1075} variation. It asked for the lactic acid acidity obtained from the actually measured lactic acid acidity and the

absorbance measured by the infrared ATR method, respectively, and was shown in drawing 6 as aging of lactic acid acidity. As shown in drawing 6, the actually measured lactic acid acidity and the lactic acid acidity measured by the infrared ATR method were in agreement, and it was checked that this invention method is applicable to control of lactic acid fermentation. Therefore, in production of fermented milk, such as yogurt, it was able to decide on the optimal time for a fermentation stop by measuring change of an infrared absorbance.

[0019]

[Work example 2]Lactic acid fermentation was carried out by 12% of reduction powdered-skim-milk concentration within a 37 ** thermostat using the same starter as Example 1. At this time, the starter's inoculation concentration was fermented under 1%, 3%, and 5% of conditions. Measurement by an infrared ATR method was performed like Example 1. The relation between obtained amendment absorbance R_{1075} and the amount of lactic acid acidity was shown in drawing 7. According to drawing 7, the relation between amendment absorbance R_{1075} and lactic acid acidity was in linear relation similarly, and it was not based on the starter's inoculation concentration, but the inclination was constant, and in agreement with the inclination moreover obtained in Example 1. It asked for the lactic acid acidity obtained from the lactic acid acidity actually measured like Example 1 using this inclination, and the absorbance measured by the infrared ATR method on the other hand, respectively, and was shown in drawing 8 as aging of lactic acid acidity. The lactic acid acidity actually measured as shown in drawing 8, and the lactic acid acidity measured by the infrared ATR method were mostly in agreement. Even if it changed the starter's inoculation concentration, it became clear that there was no influence in fermentation control. By operation of this invention method, when measuring lactic acid acidity with a titrimetric method, practical fermentation control was attained at the above accuracy of measurement.

[0020]

[Work example 3]In this example, in lactic acid fermentation, the spectrometry by this invention method replaced with lactic acid acidity measurement, and the usable thing was supported. Based on the measurement result shown in Examples 1 and 2, the correlation of the amount of absorbance variations and lactic acid acidity variation was searched for. Each measurement result was processed as follows. Lactic acid titratable acidity measured the titratable acidity before fermentation beforehand, deducted this measurement result, and made it the variation of lactic acid acidity. The infrared absorbance measured the absorbance before fermentation according to Example 1, calculated the value which amended, deducted this value from each absorbance, and made it the amount of absorbance variations. The data processed in this way was plotted like drawing 9. Both showed linearity, and when they searched for both correlation, they got the regression of the several 3 following formulas.

[0021]
[Equation 3]
乳酸酸度変化量（%）＝ -0.032 － 29.15 × 吸光度変化量

The correlation coefficient on the statistical work of this regression is 0.997.
[0022]That is, lactic acid acidity variation can be calculated from an absorbance variation. It was possible to have measured the acidity of a fermented product easily by an infrared absorbance having lactic acid titratable acidity and close correlation, and getting to know the lactic acid acidity before several 2 formulas and a fermentation start.
[0023]
[Effect of the Invention]By this invention, the fermentation control method of having used the infrared absorbance is provided. It also becomes this measuring method can be measured for a short time, and possible to use as an in-line sensor and to quantify change of the metabolite under fermentation simultaneously.

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CLAIMS

[Claim(s)]
[Claim 1]How to check change of a fermentation state and to perform fermentation control by measuring an infrared absorbance with a unique infrared-absorption wave number of an ingredient which carries out a concentration change, and measuring this absorbance or the amount of absorbance variations by a fermentation process.
[Claim 2]The fermentation controlling method according to claim 1 asking for an expression of relations of change of an infrared absorbance of a unique infrared-absorption wave number of an ingredient which carries out a concentration change beforehand by a fermentation process, changing a value of an absorbance or the amount of absorbance variations into a change control value of a fermentation state, and checking a fermentation state.
[Claim 3]The fermentation controlling method according to claim 1 or 2 an ingredient which carries out a concentration change by a metabolic turnover by a fermentation process is a substance which has specific absorption in the infrared absorption wave number field 900 - 1200 cm^{-1}.
[Claim 4]The fermentation controlling method according to claim 1 to 3 being the value which an absorbance amended from an infrared absorbance in a wave number which shows specific absorption with an infrared absorbance in the independent or multiple number of reference waves.
[Claim 5]The fermentation controlling method according to claim 1 to 4 an infrared spectrometry method is a method by an infrared total-reflection-attenuation (ATR) method.
[Claim 6]The fermentation controlling method according to claim 1 to 5 fermentation is lactic acid fermentation.
[Claim 7]The fermentation controlling method according to claim 1 to 5 fermentation is alcoholic fermentation.
[Claim 8]How to measure lactic acid acidity in lactic-acid-fermentation liquid from correlation with lactic acid acidity which measured an absorbance of 1075 cm^{-1} which amended lactic-

acid-fermentation liquid with an absorbance of 960 cm^{-1} and 1184 cm^{-1} using an infrared total-reflection-attenuation (ATR) method, and was measured beforehand.

[Translation done.]